Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

BACILLUS ABORTUS* OF BANG, THE CAUSE OF CONTAGIOUS ABORTION IN CATTLE.†

W. J. MACNEAL AND JOSEPHINE E. KERR.

(From the Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.)

THE existence of a contagious disease of cows, the most characteristic manifestation of which is the premature delivery of the fetus, has been recognized by veterinarians and by practical husbandmen for a long time. The transmissibility of this disease was proven experimentally by Brauer[1] and has been abundantly confirmed. The detection of the specific cause and its artificial cultivation has seemed to offer special difficulties, as may be judged from the painstaking but inconclusive work of Nocard[2] in France and of Moore[3] in this country. In 1897 Bernhard Bang[4] found in the uterine exudate of a cow, slaughtered during an attack of this disease before the abortion had taken place, a small short bacillus with very interesting biological properties, which he was able to grow in pure culture, and by the inoculation of which he was able to produce the disease in healthy animals. The usual methods of artificial culture failed to produce cultures of this organism. Bang and Stribolt obtained growth by inoculating the uncontaminated uterine exudate obtained after slaughter into a serum gelatin agar. The medium employed was the usual meat water peptone broth to which had been added 0.75 per cent agar and 5 per cent gelatin. This was melted and cooled to about 45° C. and then mixt with about half its volume of sterile blood serum. While still fluid, the medium was inoculated and dilutions made in a series of test tubes in the ordinary way. After mixing, the medium was solidified by immersing the tube in cold water and was then incubated at 37° C. The colonies of the bacillus developed in such tubes only in a particular zone beginning about 5 mm. beneath the surface of the medium and extending down for 1 to 1.5 cm. Below this region the medium remained clear. Bang concluded therefore that the bacillus is neither an aerobe nor an anaerobe in the usual sense, but exhibits a very peculiar behavior in respect to oxygen, requiring a partial pressure of oxygen less than that present in the atmosphere, and is unable to grow in the presence of the ordinary air, or in the absence of oxygen. This interesting feature was very fully examined by Bang and Stribolt and they found that there are two optima in the partial pressure of oxygen, one somewhat less than the partial pressure of atmospheric oxygen, and the other the partial pressure of nearly pure oxygen (about 90 per cent). A tube culture on serum gelatin agar incubated in pure oxygen or in ordinary air under a pressure of five atmospheres gave rise to two zones of colonies, one near the surface of the medium and the other near the bottom of the tube. Between them the medium remained clear. Good cultures of the bacillus were also obtained in serum glycerin broth in an atmosphere of nearly pure oxygen.

---

* The germ has been named Abortusbacillus by Bang and Bacterium abortivum by Chester (*Manual of Determinative Bacteriology*, New York, 1901, p. 121). The name Bacillus abortus has been adapted by transposition of that given by Bang. The specific name abortus, being in the genitive case, may be properly employed with either of the generic terms, Bacillus or Bacterium.

† Received for publication February 16, 1910.

[1] Cited by Ostertag, Kolle und Wassermann, *Handbuch*, 3, p. 825.

[2] Cited by Bang, *Ztschr. f. Thiermedicin*, 1897, 1, p. 241.

[3] *Amer. Vet. Méd. Assoc. Rep. of E. V. Wilcox*, Exper. Sta. Rec., 1904–5, 16, p. 122.

[4] *Op. cit.*

With these cultures Bang has been able to produce the disease in cows by application to the vaginal mucous membrane, by intravenous injection, and by feeding. He has also infected sheep, goats, rabbits, and one mare, producing abortion and recovering the bacillus from the uterine exudate in each case.

Nowak[1] has confirmed the work of Bang, and placed practically beyond question the etiological relation of the bacillus to the disease. The particular advance which he has made, however, is the discovery of a practicable plate method for the isolation of the abortion bacillus from contaminated material. For this purpose ordinary agar is melted and cooled to 50° C., then mixt with about one-fourth its volume of naturally sterile blood serum, and poured into sterile Petri dishes where it is allowed to solidify. The piece of the placenta or other material from the abortion is now streaked over several of these plates in succession, according to the usual bacteriological technic for obtaining streak dilution cultures. The plates are then placed at 37° C. for 24 hours to allow contaminating aerobic bacteria to

[1] *Ann. de l'Inst. Pasteur*, 1908, 22, p. 541.

develop. Parts of the plate surface free from colonies are now marked with a wax pencil, as it is upon these areas that the growth of the abortion bacillus may subsequently appear if it is present. The plates are next put into a glass jar or desiccator together with a culture of the common hay bacillus, B. subtilis. About 1 sq. cm. of culture surface of the latter organism should be allowed for each 15 c.c. air capacity of the jar. The jar is now closed and placed at 37° C. for three days, at the end of which time the transparent colonies of the abortion bacillus will have developed, if it is present. The growth of the B. subtilis culture serves to absorb the oxygen in the jar to just the partial pressure required for the development of the abortion bacillus.

It may be accepted as certain that this germ is the microbic cause of contagious abortion in several species of domestic animals, but it is too early to conclude that it is the only germ causing a disease in which abortion is a prominent feature. In fact, Ostertag[1] has investigated an epidemic of contagious abortion in mares, in which he was unable to find this bacillus, but did find a streptococcus in every case.

In the United States, contagious abortion is a widespread disease of cattle, and recent investigations of the disease at the Arizona Agricultural Experiment Station have been reported by Wilson, and at the Connecticut Storrs Agricultural Experiment Station by Beach. So far the isolation of the bacillus of Bang appears not to have been reported in this country. In reporting our work at this time we purpose to add further confirmation to the work of Bang and to direct the attention of American bacteriologists to the very interesting biological properties of this bacillus discovered by him.

Case 1.—Cow. Abortion occurred at 5 A.M. July 10, 1908, at beginning of seventh month of pregnancy. The placenta was removed from the uterus by the herdsman and arrived at the laboratory at 10:30 A.M. Many of the cotyledons were yellowish-white in the centers, some were entirely broken down and purulent on the surface. A large area of the amnion was thickened and contained many small hemorrhages, and a few opaque yellowish areas. A small piece of purulent cotyledon was suspended in sterile broth and two small guinea-pigs (not pregnant) were inoculated, one subcutaneously, the other intraperitoneally. Neither guinea-pig was seriously affected.

Case 2.—Cow. Abortion, during the eighth month of pregnancy, occurred August 30, 1908. A portion of the placenta was kept in a refrigerator and examined on

---

[1] *Op. cit.*

September 3. The appearance of the placenta resembled that of Case 1. There was no offensive odor. Bits of the altered placental tissue were inoculated into blood broth fermentation tubes, and blood agar, blood gelatin, and plain agar stab cultures were made. All these cultures were incubated at 37° C. for 24 hours and by microscopic examination were found to contain a variety of bacteria. They were kept in the refrigerator until September 7 upon which day two pregnant female guinea-pigs were inoculated. The first received three drops of a blood broth fermentation tube culture subcutaneously. Nine days later, September 16, edema of the vulva was observed for the first time. On September 23 there was a purulent discharge at the vaginal outlet but the fetuses were still present in the abdomen. On September 25, two days later, the uterus was empty, there was no vaginal discharge apparent, and no sign of the products of conception in the cage. The animal was killed by chloroform and blood broth fermentation tubes were inoculated from the inside of the partly involuted uterus. These remained free from growth. The second guinea-pig received on September 7 three drops of the same blood broth culture into the vagina. No edema of the vulva or other pathological change was observed in this animal. On September 25, Caesarian section was performed under ether and two living and almost fully developed fetuses were removed. Fermentation tubes of blood broth were inoculated with exudate from the endometrium, with the amniotic fluid, and with bits of placental tissue. These gave no growth. Direct microscopic examination of the tissues was negative.

Case 3.—Heifer. Abortion occurred during the eighth month of pregnancy on March 17, 1909. A piece of the placenta was examined; it presented the appearance described in Case 1. Bits of the altered placental tissue were used to make streak inoculation on a series of six serum agar plates, according to the method of Nowak. Two stab cultures with Stribolt's serum gelatin agar were also made, and a pregnant female guinea-pig was inoculated subcutaneously with 0.5 c.c. of a suspension of a bit of placenta in broth. This guinea-pig aborted during the night of March 27 but none of the products of conception were found and no further work was done with the animal. Direct microscopic examination of cover-glass preparations of the cow's placenta showed few bacteria, but at least two different kinds were present, a ong bacillus and a very short bacillus.

The serum agar plates (Nowak) were incubated at 37° C. in the air for 24 hours and the colonies which developed were checked by a blue pencil mark on the outside of the bottom of the Petri dish. The dishes were then placed in a Novy anaerobe jar together with one Petri dish plate culture of B. subtilis,[1] the jar closed, and incubated four days at 37° C. On March 22 excellent colonies corresponding to the description and to the photographs of Nowak were found in rows on the portions of the plates formerly free from colonies. The colonies were made up of short non-motile bacilli without spores. Subcultures were made on inclined serum agar tubes, some of which were incubated in the air and others in the glass jar together with plates of B. subtilis. After three days the former set of tubes showed no growth, and on the latter excellent cultures had developed. Subcultures were again made from these, the remainder of the growth was suspended in sterile broth, and 0.5 c.c. of the suspension was injected subcutaneously into each of two pregnant female guinea-pigs (March 25). One of the guinea-pigs inoculated aborted during the night from March 28 to 29, only three and a half days after inoculation. The two fetuses were of nearly full size but both were dead. No placenta was found. At autopsy it was found that one of the

[1] Inoculated by overflowing the surface of the solidified agar with a broth suspension of the bacillus.

young guinea-pigs had not breathed, but in the other one the lungs were well expanded, the stomach filled with gas, and there was some gas in the upper part of the small intestine. The animal must therefore have lived a short time after birth. Serum agar plates were inoculated with the heart blood and the intestinal contents of each fetus but none of the specific bacteria were detected. The mother guinea-pig was chloroformed at 11 A.M. and autopsy performed at once. At the point of inoculation there was a hard, almost cartilaginous infiltration, the size of a quarter, and the skin over it was red in color. On section the subcutaneous tissue was yellowish-white in color and very firm. The bacillus of Bang was detected here in pure culture, microscopically, and by plate culture. Cultures inoculated with heart blood and with the serous fluid from inside the uterus remained sterile. In this animal, then, aborting 3½ days after subcutaneous inoculation with the bacillus, there was no proof that the infection had extended to the uterus.

The other pregnant female guinea-pig, inoculated subcutaneously on March 25 with a pure culture of the bacillus, aborted early on the morning of April 2, eight days after inoculation. At 8:00 A.M. two half-grown fetuses and two placentae were found in the cage with her, one of the latter having been partly eaten. B. abortus was found in each placenta by microscopic examination of cover-slip preparations, and also by plate cultures. It was not found upon the plates made from the fetal livers. The mother was chloroformed at 8:30 A.M. and autopsy performed at once. At the point of inoculation the skin was reddened and there was a very hard subcutaneous swelling. Upon section the corium was greatly thickened and infiltrated with pus, walled off below by a dense capsule of cartilaginous consistency. The bacillus was found here in pure culture by microscopic examination and by plate cultures. The bacillus was not found in the heart blood, but typical colonies developed on the plate cultures inoculated with uterine exudate.

From this cow's placenta (Case 3), then, was obtained a bacillus corresponding to the description of Bang and of Nowak, and by inoculations with a pure culture of this germ, premature labor was induced in guinea-pigs and the germ was recovered from the placenta and from the endometrium of one of these animals.

Case 4.—Heifer. Calf was still-born at term, April 9, 1909. The placenta and the body of the calf were examined, and a number of plate cultures made from them, but no B. abortus was isolated.

Case 5.—Cow. Living calf was born at term, April 12, 1909. Placenta was not obtained. A sample of vaginal discharge was brought to the laboratory April 14 and plate cultures made. B. abortus could not be isolated.

Case 6.—Heifer. Abortion occurred at fifth month on April 15, 1909. The animal had been carefully examined on the previous day before the class, and appeared perfectly healthy. Complete membranes and fetus were brought to the laboratory. B. abortus could not be isolated.

Case 7.—Cow. Abortion occurred April 21. Only a piece of amnion was saved for examination; no placental tissue. B. abortus could not be isolated.

Case 8.—Cow. Normal calf was born at term, May 3, 1909. Placenta was brought to the laboratory. It appeared normal. Plate culture failed to isolate B. abortus.

Case 9.—Cow. Normal calf was born at term, May 5, 1909. Placenta was not found. Vaginal discharge was examined by the plate method with negative results.

Case 10.—Cow. Premature delivery of a living calf occurred July 9, 1909, at the beginning of the eighth month of pregnancy. Placenta was not obtained. Some of the discharge collected from the ground in an open field was brought to the laboratory. B. abortus could not be isolated by the plate method.

Case 11.—Cow. Abortion, at seventh month of pregnancy, occurred July 10, 1909. A piece of placenta was brought to the laboratory the next morning. Most of the cotyledons were grayish yellow and purulent. Five serum agar plates (Nowak method) were made, and on two of these pure colonies of B. abortus were obtained. The bacillus is identical in cultural characteristics with that obtained from Case 3. No animal inoculations have been made with it.

Case 12.—Pig. Sow aborted July 18, 1909. Two fetuses and a piece of the membrane, but no placental tissue, were brought to the laboratory. B. abortus could not be isolated.

Case 13.—Pig. Sow aborted during the night from July 28 to 29. One fetus and a piece of membrane with a bit of broken down placental tissue was brought to the laboratory July 29. B. abortus could not be isolated.

Case 14.—Cow. Living calf was born at term, September 24, 1909. Placenta was examined. B. abortus could not be isolated.

Case 15.—Cow. Living calf was born at term, September 25, 1909. Placenta was examined. B. abortus could not be isolated.

Case 16.—Cow. Living calf was born at term, September 29, 1909. Placenta was examined. B. abortus could not be isolated.

Case 17.—Cow. Living calf was born at term, October 3, 1909. Placenta was examined. B. abortus could not be isolated.

Case 18.—Cow. Living calf was born at term October 11, 1909. Placenta was examined. It was normal in appearance. B. abortus could not be isolated.

Case 19.—Cow. Living calf was born at term, November 8, 1909. Placenta was examined. B. abortus could not be isolated.

SUMMARY.

Of the 19 cases examined two were sows and 17 were cows. Of these latter, 10 were delivered at term. In the other 7 delivery was premature. Of these, one case, No. 6, was clinically not an abortion due to infection. This leaves six cases which were clinically cases of contagious abortion. Two of these, Cases 1 and 2, were examined before the plate method was employed, and cultures were negative. A guinea-pig inoculated from Case 2 aborted. Of the four remaining cases, No. 3 and No. 11 gave positive cultures of B. abortus from the placenta in each case. In Case 7 only a bit of membrane without placental tissue was available and in Case 10 only some of the discharge gathered from the ground. Both of these, examined by the plate method, gave negative results.

Subcutaneous inoculation into a pregnant female guinea-pig, of a broth culture of the mixt bacteria of a contaminated placenta (Case 2),